Alteration of Oligosaccharide Biosynthesis by Genetic Manipulation of Glycosyltransferases[a]

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INTRODUCTION

Carbohydrate chains can directly mediate or modulate the function of glycoproteins in diverse biological processes.[1] The ability to manipulate the oligosaccharide structures of glycoproteins in order to alter their biological properties would be of obvious value. This is particularly relevant with regard to biologically important molecules such as growth factors, hormones, and other therapeutic agents that are being produced in cultured cells. Altering the sugar chains of these glycoproteins may improve their therapeutic value by increasing their efficacy, altering their circulatory half-lives, and/or increasing their target specificity. In addition, altered glycosylation of cell-surface components may provide insight to the precise roles that cell-surface glycoconjugates play in processes such as migration, adhesion, development, and malignancy.

Several methods have evolved to alter glycosylation in cells. These have included the use of reagents that inhibit glycosylation as well as inhibitors of glycosylation processing.[2] These inhibitors have been widely used to study the sugar chains of glycoconjugates, but many of these reagents are toxic to cells, and in some instances, their effects are only partial. Another approach has been to use mutagenized cells that are resistant to the toxic effects of specific lectins due to deficiencies in corresponding glycosylation reactions.[3] One limitation of this approach is that mutants are not generated at each step of the biosynthetic pathway. In addition, both of these approaches are of limited use for obtaining large, complex oligosaccharides, inasmuch as they result in the formation of incomplete or truncated carbohydrate structures.

One method that overcomes these limitations and that allows one to selectively manipulate oligosaccharide structure is to express cloned genes for glycosyltransferases into mammalian cells. As more genes for these enzymes are cloned, the possibilities for altering the biosynthetic pathways of oligosaccharide in cells could be substantial. It is this new approach that will be the focus of this review.

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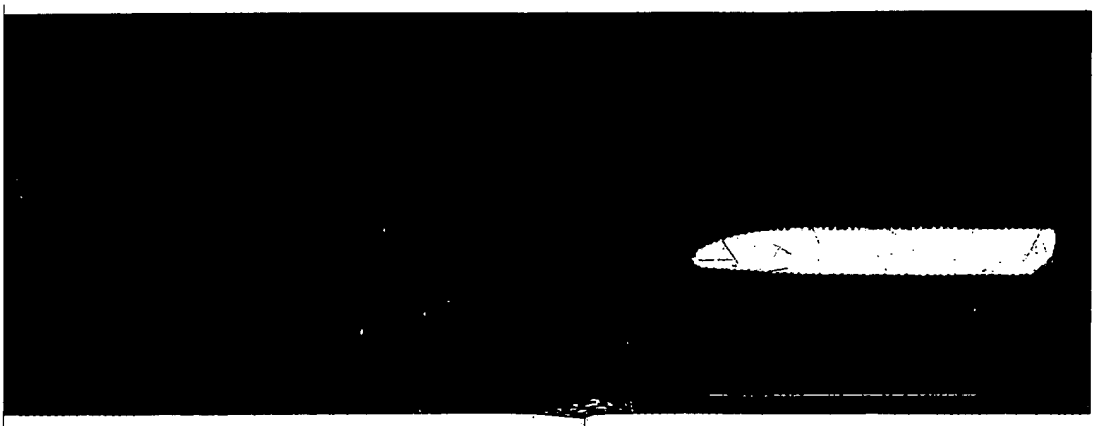

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alized to the endoplasmic reticulum and
se enzymes catalyze the transfer of sugars
ermediates to mono- and oligosaccharide
different glycosyltransferases have been
e of these enzymes share common sub-
mology. The topology and domain struc-
s, however, are remarkably similar in that
ontaining short cytoplasmic domains and
ailability of cDNAs for many of these
them into host cells and to subsequently

netic manipulation of glycosyltransferases
xpression of glycosyltransferases that are
ogenous glycosyltransferases), and over-
cells containing endogenous activities of
ferases). The first approach has been the
g for alterations in glycosylation is rela-
ovel oligosaccharides on the cell surface
second approach, on the other hand, re-
because increasing the levels of glycosyl-
rate different types, but rather, different

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osyltransferases

n of exogenous glycosyltransferases has
ation, such as fucosylation and sialylation.
ies that have used this approach to modify

catalyzes the transfer of fucose (Fuc) from

fucose to galactose (Gal) residues,
antigen. Similar to the studies with
α1,2 FT into COS-1 cells, which l
of H blood group antigen.[10]

C. α1,3 Galactosyltransferase
UDP-Gal to terminal Gal residues
GT into CHO cells results in the
rides.[11] Furthermore, the expressio
in terminal sialylation of sugar chai
competition between different glyc

D. α2,6 Sialyltransferase (α2,
from CMP-SA to terminal Gal re
protein into CHO cells results in
chains.[12] As was the case for exan
between the transfected sialyltransf
SA in an α2,3 linkage to Gal.

E. β1,3 Galactosyltransferase (
GlcNAc residues in the biosynthes
mine chains. This class of oligosac
importance because it serves as the
Transfection of the cDNA encodin
the biosynthesis of type 1 *N*-ace
addition, many of these chains we
contrast to the studies described
expression of glycosyltransferase
during elongation) can also alter

Endogen

To date, the only example of
glycosyltransferase is that of β1,4
lyzes the transfer of Gal from UI
synthesis of type 2 (Gal β1,4 Glc
complex oligosaccharides. β1,4 G
in that one gene encodes two simil

fucose to galactose (Gal) residues, resulting in the formation of the H blood group antigen. Similar to the studies with α1,3 FT, the introduction of the cloned gene for α1,2 FT into COS-1 cells, which lack this enzymic activity, results in the formation of H blood group antigen.[10]

C. α1,3 Galactosyltransferase (α1,3 GT) catalyzes the transfer of Gal from UDP-Gal to terminal Gal residues of oligosaccharide chains. Transfection of α1,3 GT into CHO cells results in the expression of α1,3 Gal-containing oligosaccharides.[11] Furthermore, the expression of this enzyme results in a concomitant decrease in terminal sialylation of sugar chains in these cells. Thus, this study also shows that competition between different glycosyltransferases can affect glycosylation *in vivo.*

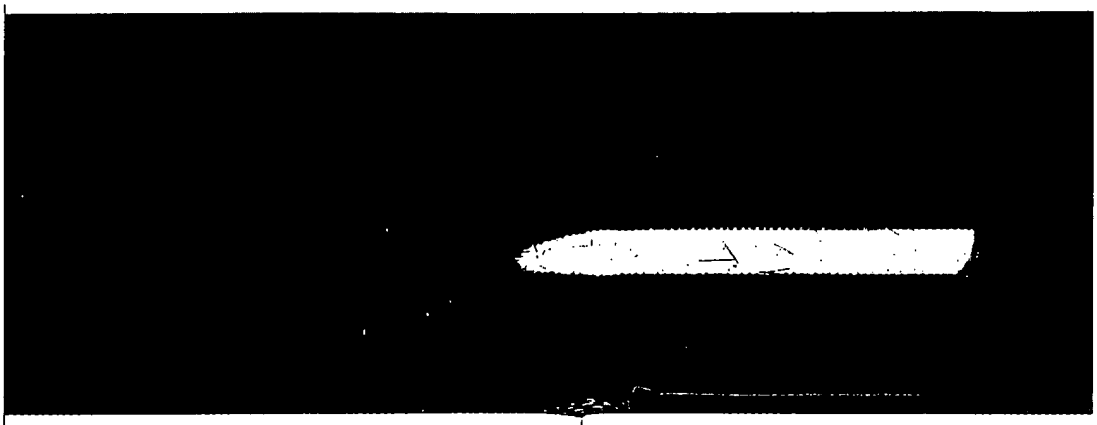
D. α2,6 Sialyltransferase (α2,6 ST) catalyzes the transfer of sialic acid (SA) from CMP-SA to terminal Gal residues. Transfection of the cDNA encoding this protein into CHO cells results in the expression of appropriately sialylated sugar chains.[12] As was the case for example C above, there is competition for substrates between the transfected sialyltransferase and a similar endogenous enzyme that adds SA in an α2,3 linkage to Gal.

E. β1,3 Galactosyltransferase (β1,3 GT) catalyzes the transfer of Gal to terminal GlcNAc residues in the biosynthesis of type 1 (Gal β1,3 GlcNAc) *N*-acetyllactosamine chains. This class of oligosaccharides is of particular biological and structural importance because it serves as the core structure for various blood group activities. Transfection of the cDNA encoding this enzyme into human colonic cells results in the biosynthesis of type 1 *N*-acetyllactosamine-containing oligosaccharides.[13] In addition, many of these chains were further modified with SA and Fuc residues. In contrast to the studies described above, this study is unique in showing that the expression of glycosyltransferase acting early in oligosaccharide biosynthesis (*i.e.*, during elongation) can also alter glycosylation.

Endogenous Glycosyltransferase

To date, the only example of over-expression of an endogenously expressed glycosyltransferase is that of β1,4 galactosyltransferase (β1,4 GT). β1,4 GT catalyzes the transfer of Gal from UDP-Gal to terminal GlcNAc residues in the biosynthesis of type 2 (Gal β1,4 GlcNAc) *N*-acetyllactosamine cores of all *N*-linked complex oligosaccharides. β1,4 GT is unique among the cloned glycosyltransferases in that one gene encodes two similar forms of the enzyme that differ by an additional 13 amino acids at the cytoplasmic N-terminus of the long form that is not present in the short form of the enzyme.[14,15] Both the long and the short form are localized primarily in the Golgi complex. However, the long form of GT is also targeted to the plasma membrane,[15] where it associates with the cytoskeleton and functions as a cell-adhesion molecule.[16]

Because of the central role this enzyme plays in the biosynthesis of *N*-acetyllactosamine–containing oligosaccharides, the effects of overexpressing this enzyme were explored in detail. Transfection of the cDNAs encoding the two forms of β1,4 GT into F9 embryonal carcinoma cells expressing endogenous enzyme results in a threefold increase in total β1,4 GT activity compared to control cells.[17] Analysis of [^{3}H]Gal-labeled glycoproteins and glycopeptides by a variety of methods revealed



ifferences in glycosylation. Similar anal-
mal-associated membrane glycoprotein
ycosylation between the transfected and
ted β1,4 GT to affect glycosylation was
inasmuch as an excess of substrate was
and exogenous GT. The transfected GT
ex, and, more importantly, were elevated
. Thus, in these cells, β1,4 GT is not rate

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ctures through genetic manipulation of
ity. It is apparent that this technique has
structure when an exogenous enzyme is
this enzyme is responsible for a terminal
study has examined the effects of over-
rase, in which there was no detectable
e still other key regulatory biosynthetic
d β1,3 GlcNAc transferase, whose over-
of these enzymes are required for the
lymers of *N*-acetyllactosamine disaccha-
correlates with increased expression of
gene encoding GlcNAc transferase V has
lls and characterization of the resulting

osaccharide structures could involve the
sferase into cells to ensure the availability
, the disruption of specific glycosyltrans-
n could be used to eliminate competing
substrate.

is directly dependent upon the presence
other factors also contribute to glycosyla-
a glycoprotein through the endoplasmic
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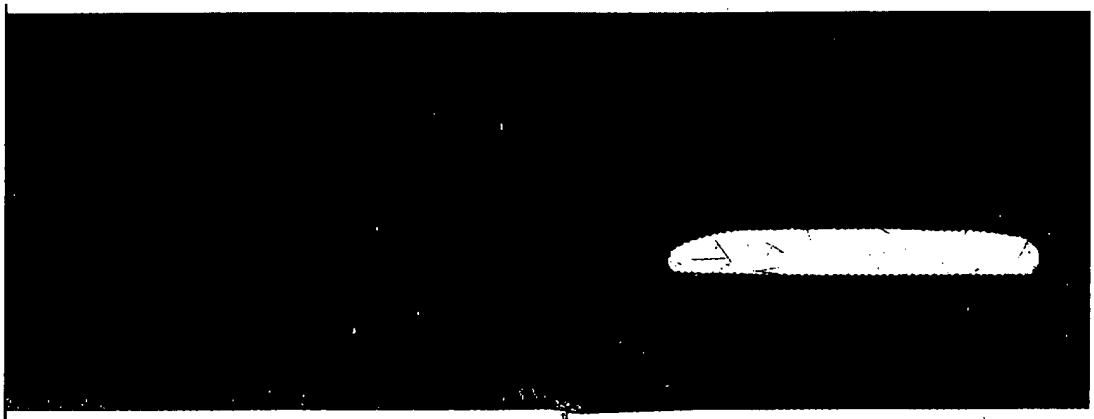

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